Reference: *Biol. Bull.* **195:** 30–42. (August, 1998)

Transport Pathways in the Neotropical Sponge *Aplysina*

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Abstract. Strands of cells distinct from the rest of the tissue were found running lengthwise through the endosome of four species of the sponge *Aplysina*. Although the strands were more highly pigmented than the adjacent tissue and could be removed intact with forceps, ultrastructural studies revealed no obvious barrier separating the cells in the strands from the rest of the tissue. The strands consist of stretches of elongate cells tightly aligned along densely bundled collagen fibrils, and areas of other elongate cells that possess numerous filopodia. When sponges were fed fluorescent latex beads *in situ*, beads were taken up and transported specifically into the strands; eventually they were found at the tip of the sponge and further down the stalk, away from the site of feeding. Beads injected into endosomal strands were also transported upwards in the strands to the tip of the sponge. Video microscopy of cells in strands that had been exposed along a portion of their length showed no bulk movement of cells; but individual cells were seen moving in both directions along the strands at 0.025–0.04 $\mu m \cdot s^{-1}$. The endosomal cell strands are suggestive of a primitive nutrient transport pathway in sponges.

Introduction

Sponges were the first multicellular animals to arise from unicellular protists during early metazoan evolution, at least 600 million years ago (Steiner *et al.*, 1993; Reitner and Mehl, 1995), and their tissue organization, accordingly, is quite simple. Sponges are diploblastic metazoans that possess no organ systems; their tissues consist of a limited number of cell types that surround and penetrate a mesohyl of collagen. The entire animal is organized around canals and chambers through which water is channeled for feeding and respiration.

Within the Porifera, however, some groups differ markedly from this fundamental plan. The Hexactinellida, for example, are mostly syncytial (Reiswig, 1979; Mackie and Singla, 1983), a condition that allows symplastic transport of food (Perez, 1996; Wyeth *et al.*, 1996) and rapid conduction of action potentials that control the feeding current (Leys and Mackie, 1997). The Cladorhizidae, on the other hand, are deep-sea demosponges that are effectively carnivorous and lack flagellated chambers, which are otherwise a key poriferan character (Vacelet and Boury-Esnault, 1995). In a group as old as the Porifera, other exceptions to the basic sponge plan probably exist.

One intriguing possibility lies within demosponges of the genus *Aplysina*. These sponges, which are known as bacteriosponges for the vast number of bacteria living within their mesohyl, belong to the Verongida, an order that contains numerous pharmaceutically interesting chemicals (Bergquist *et al.*, 1991; Ciminiello *et al.*, 1994, 1997). An unusual feature of some sponges in this genus is the presence of longitudinal strands of elongate cells in the endosome. The strands, up to 100 μm in diameter and particularly densely populated by bacteria, constitute a quasi-tissue that runs vertically through sponges of tube, chimney, and rope-form alike.

Despite their prominence in these sponges and their marked absence in other members of the phylum studied to date, the strands have received only passing mention in the literature. Tsurumi and Reiswig (1997) suggested that the "endosomal" tissue strands in *Aplysina cauliformis* (a rope-form sponge) serve as migration routes for rapid transport of cells for tip growth. Similar elongate cells, in the endosome and around the water canals in *Verongia* (synonymous with *Aplysina*), were suggested

Received 30 March 1998; accepted 1 June 1998.

to be contractile cells that act together to control water flow through the canal system (Vacelet, 1966). To have tested either of these hypotheses would have required cell labeling studies and, if possible, video-enhanced-contrast microscopy of live tissue, techniques that were not readily available until recently. We have now undertaken both approaches and present here a detailed description of the endosomal tissue strands and their role in transport of materials.

Materials and Methods

Description of the sponges and collection site

Aplysina cauliformis (probably synonymous to *A. fulva*), a neotropical rope-form sponge, grows at the tip (Wulff, 1990), reaching about 1 cm in diameter, but up to several meters in length. It is pink or purple on the outside and yellow inside. At depths of 7–10 m, *A. cauliformis* is typically branched and grows only 20–30 cm in height. *Aplysina lacunosa* is a mustard-yellow tube-form sponge. The tubes are 10–50 cm in length and can occur singly, but more often several of them bud from the same base. The tubes are 5–10 cm in diameter with an atrial diameter of 2–5 cm. *Aplysina rigida,* a branching stick-form sponge, is pink outside and yellow on the inside, but is both thicker (up to 5 cm in diameter) and taller (up to 1 m in height in 7-m-deep waters) than *A. cauliformis. Aplysina fistularis* is a robust, branching, stick-form sponge in Barbados, but it may be tube-form in other habitats in the Caribbean. *A. fistularis* is yellow outside and has an irregular outer surface, but it lacks the lacunae characterizing *A. lacunosa*; it is 3–5 cm in diameter and reaches 20–30 cm in height at depths of 7–10 m.

Species of *Aplysina* were manipulated *in situ* and collected by scuba from coral rubble at a depth of 7–10 m off the Bellairs Research Institute, St. James, Barbados (13°10′N, 59°40′W).

Field experiments

Latex bead feeding. Plastic bags (4 × 7 cm) open at both ends were slipped over the branches of *A. cauliformis in situ* and, about 5–10 cm from the top, were gently secured at both ends with plastic twist ties (Figs. 1 and 2a). A plastic syringe and needle were used to inject the bag with 5 ml of 1-μm fluorescent latex beads (Molecular Probes, Eugene, OR) diluted 1:5 with seawater. The beads were not treated with serum to prevent clumping, because trial experiments showed that clumping did not interfere with bead uptake. The bead-filled bags were left on the sponges for 4 h and then removed; the beads had been cleared from the bags during this period. Two branches were collected for fixation at 4 and 24 h, and 2, 3, 7, 11, and 13 days after feeding. The sponges were cut off at a point 15 cm from the top, placed in a plastic bag, and taken to the laboratory, where each piece was cut into three segments: the top 5 cm (Tip), the middle 5 cm where the bag had been secured (Middle), and the bottom 5 cm (Lower) (Fig. 1). The segments were fixed in 2% paraformaldehyde in seawater and placed in the refrigerator at about 4°C. After 4 hours the fixative was poured off and replaced with fresh, cold 2% paraformaldehyde.

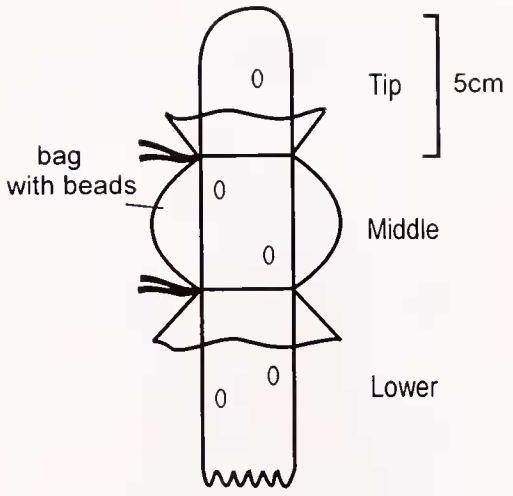


Figure 1. Diagram of a branch of *Aplysina cauliformis* showing the attachment of a plastic bag that is used to feed latex beads to the sponge. Also shown are the three segments (Tip, Middle, and Lower) that the branch was cut into; the segments were used in determining the fate of the beads.

Insertion of latex beads. Whole branches of *A. cauliformis* attached to loosely buried coral rubble were collected in plastic bags (with the coral) and brought into the laboratory; the sponges were never removed from seawater. The sponge branches were cut 7 cm from the tip, but not quite in two; thus a hinge of tissue still connected the tip segment to the main stem. The sponge was placed in a large bucket of seawater and, with the aid of a dissecting microscope poised over the bucket, 1-μm latex beads were injected with a plastic syringe and needle onto a severed cell strand and its immediate area. The wound was sewn together with dental floss and the sponges were returned to the field no more than 4 h after collection and reattached with plastic cable ties to other pieces of coral rubble. Preliminary experiments showed that, despite wave action, the wound healed and new tissue grew over the dental floss within 1–2 days. Sponges treated in this way were recollected 1, 2, 3, 7, 11, and 13 days after wounding, cut into three segments as described above, and fixed in 2% paraformaldehyde in seawater.

Laboratory analysis of field experiments

Sponge pieces were transported while still in fixative to the University of Victoria, British Columbia, Canada, where

they were rinsed in phosphate-buffered saline (PBS) for 4 h, infiltrated overnight in 1:1 PBS and Tissue Tek (O.C.T. Compound 4583, Sakura Finetechnical, Tokyo), and embedded in 100% Tissue Tek by freezing in liquid nitrogen. Blocks of embedded tissue were stored at −20°C. Longitudinal sections (30 μm) from each segment (Tip, Middle, and Lower) of one of the two branches from each collection period were cut on a cryomicrotome. Sections were mounted on slides coated with poly-L-lysine and stored at −20°C. Sections were viewed with a Leitz Aristoplan epifluoreseence microscope equipped with the FITC filter (450–490-nm excitation) through which the tissue appeared green and orange and the latex beads bright yellow. Latex beads were counted in 10 sections chosen at random from each segment of the sponge for every time interval of collection. The location of the beads was scored as follows: i) in the tissue (*i.e.*, in or near the flagellated chambers) of the sponge; ii) at the edges of water canals; and iii) in cell strands. Where beads were too numerous to count (for example in the pieces collected within the first 24 h after treatment), 100 beads were first counted, and estimates of every hundred beads visible thereafter were made. Selected sections were later stained in hematoxylin and eosin but not cleared in xylene, and then mounted in PBS-glycerol. This procedure allowed us to see the location of beads within the strands.

Video microscopy

Pieces of *A. cauliformis* were transferred to flow-through seawater tanks at the Bellairs Research Institute without removal from seawater. A 4-cm piece of a sponge branch was cut in two lengthwise with a sharp disposable scalpel. Where a strand was exposed without damage along a portion of its length, the piece of branch was tied with dental floss to a glass microscope slide with the cut surface against the slide. With the exposed strand facing upwards, the slide was rested on supports at either end in a flow-through perfusion chamber so that the sponge did not touch the bottom of the dish. The perfusion chamber was set on the stage of a Zeiss compound microscope and the strands were filmed with a 10× objective lens for up to 5 h using a Panasonic CCD color video camera; the image was digitally enhanced with an Omnex real-time digital image processor (Imagen Corp.) and recorded on Fuji video tapes in real time with a Panasonic VCR. Photographs were taken from the monitor of a Technitron television with a Nikon FG camera on TMAX 400 ASA film.

Bright field microscopy

Pieces of *A. cauliformis* were cut in half lengthwise to expose the tissue strands and placed strand-side up, in a 5-cm diameter dish of seawater, on the stage of a Leitz Orthoplan microscope. The exposed surface was illuminated with two adjustable microscope lamps, and photographs were taken on 100 ASA Fujicolor print film.

Electron microscopy

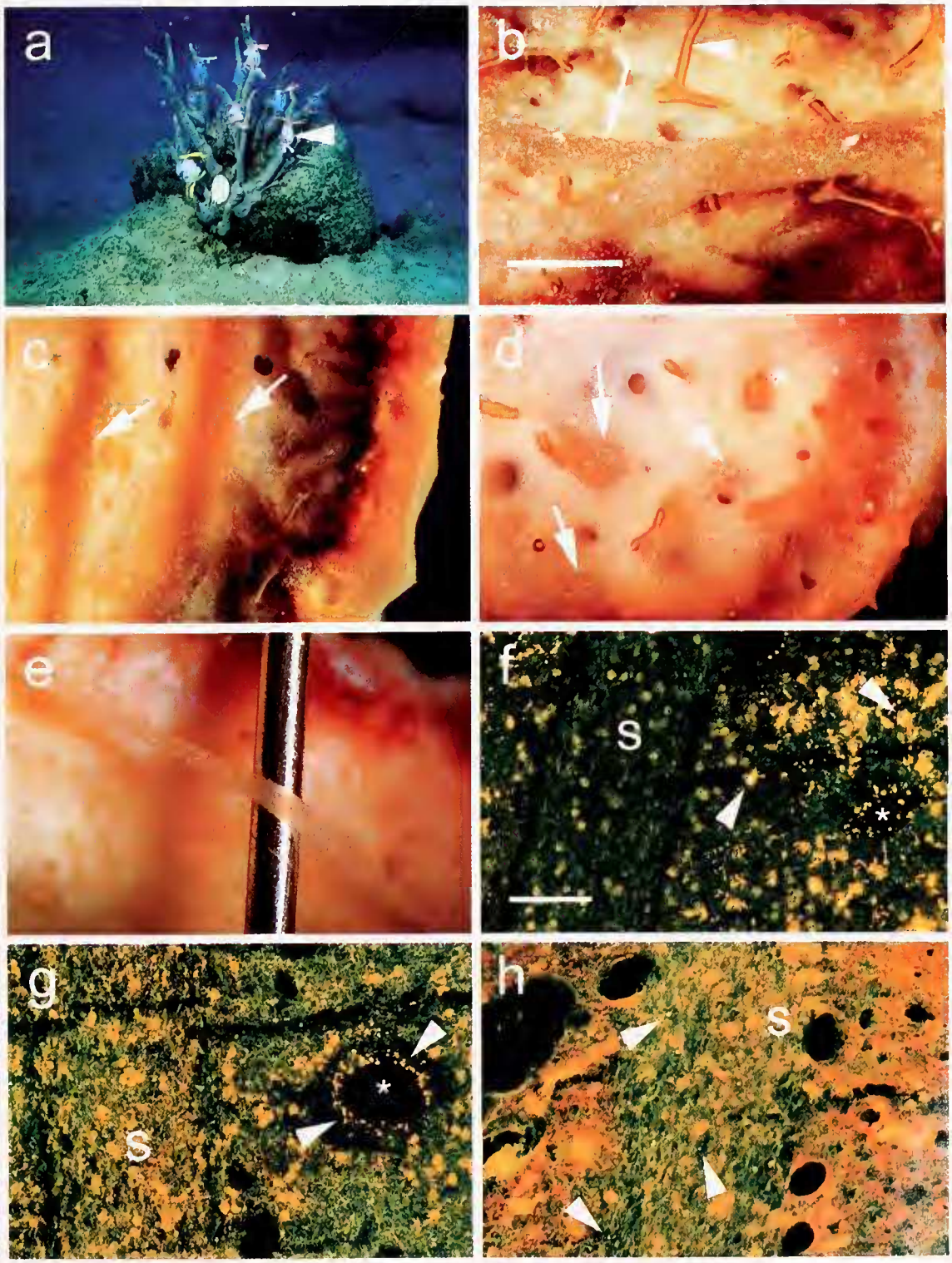
Tissue strands were dissected, with a scalpel and fine forceps, out of freshly collected sponge branches. Strands and whole pieces of sponge with exposed strands were fixed in a cocktail of cold 1% osmium tetroxide and 2% glutaraldehyde in 0.45 *M* sodium acetate buffer, pH 6.4, and 10% sucrose in the final volume. Pieces were kept in the refrigerator at 4°C for 4 h, and the fixative was then changed. After 16 to 20 h, the fixative was poured off, and the tissue was rinsed in 0.45-μm Millipore-filtered seawater and stored in 70% ethanol for transport to the University of Victoria, British Columbia. Tissue strands and pieces of sponge were then further dehydrated to 100% ethanol, stained *en bloc* in 0.5% uranyl acetate overnight at the 80% ethanol stage, rinsed three times in propylene oxide, infiltrated overnight in 1:1 Epon (Taab 812) and propylene oxide, and embedded in 100% Epon at 60°C. Silver sections were cut on a Reichert ultramicrotome with a diamond knife, stained with lead citrate, and viewed in a Hitachi 8000 transmission electron microscope.

Figure 2. (a) *Aplysina cauliformis* attached to coral rubble, with plastic bags (arrowhead) filled with latex beads attached to each branch. Latex beads were "fed" to the sponge branches in the plastic bags, and the fate of ingested materials was determined. (b–e) Live tissue strands of *A. cauliformis*. Bar: 1 mm. (f–h) Latex beads in longitudinal sections of preserved sponge branches. Bar: 50 μm. (b) A strand in a longitudinal section of a sponge. The strand (arrow) is darker than the adjacent tissue and divides to go around the spongin skeleton. The arrowhead indicates a piece of the broken spongin skeleton. (c) Two exposed strands (arrows) in a longitudinal section are similar in color to the tissue at the outer layer of the sponge. (d) A cross section of a sponge branch showing three strands (arrows), which are easily identified by their pink color and by their slight separation from the neighboring tissue. Polychaete worms (*) are an abundant macrosymbiont in these sponges and either use water canals or fashion their own pathways through the sponge. (e) A tissue strand can be lifted up with a dissecting needle, indicating the integrity and slight elasticity of the strands. (f) Four hours after feeding, latex beads (arrowheads) were well distributed throughout the tissue but were not in the strands (s). (g) Two days after feeding, the beads (arrowheads) were predominantly in the tissues around the water canals (*); strands (s). (h) Eleven days after feeding, the beads (arrowheads) were only found in the tissue strands (s) of the tip segment of the branches. Very few were found in other tissues of the sponge at this time.

Results

Description of the strands

The tissue of *A. californis* contained longitudinal strands running from the base to the tip of the sponge (Fig. 2b). The strands were darker than the adjacent tissue, and were flecked here and there by white cells that appeared to be highly refractile. The strands were most similar in color to the tissue at the cortex (Fig. 2c) and the tissue at the tip of the sponge. Up to 12 strands could be identified in any one cross section of a sponge branch (Fig. 2d). Strands ranged in diameter from 20 to 100 μm,



but the diameter of any one strand was rather uniform for its entire length. Strands could be traced for at least 3 cm and often for more than 6 cm along a sponge branch. The strands tended to be straight, with only slight bends and turns where they coursed around the spongin skeleton (*e.g.*, Fig. 2b), and there were no cross-connections between strands. Typically, strands branched or forked at the tip of the sponge, and if a strand was located along the cortex or edge of the sponge, a branch was often directed towards the cortex.

The strands were sufficiently separate from the rest of the tissue in the sponge that they could be lifted by one end with forceps and gently pulled free from the sponge. The strands were slightly elastic, as can be seen by the ability of a dissecting needle to pull a portion of a strand away from the sponge tissue (Fig. 2e). However, more than a moderate pulling resulted in the breakage and slow curling of the excised ends. Occasionally, when they were pulled out of the sponge, the strands frayed at the edges and pieces at the edges were left behind. Other parts of the sponge lacked this integrity and could not be removed intact from the sponge.

Examination of the live tissues of *A. lacunosa*, *A. fistularis*, and *A. rigida* revealed tissue strands in all three species. Strands were always distinctly more pink than the surrounding tissue, up to 100 μm in diameter, and traceable for at least 3 cm, and often for the full length of the sponge. Sections 1-cm thick cut with a scalpel from top to bottom of these sponges revealed fewer strands at the base than at the middle or tip of the sponge, and none of the strands were especially close to the cortex. In one section of *A. lacunosa*, 24 different strands were identified. Most of the strands could be followed for 4 cm or more along the length of the sponge.

Electron microscopy

Ultrastructural examination revealed no clear barrier separating the tissue strands from the rest of the sponge tissue. Only in one instance was a string of connected cells 60-μm long found at the edge of the strand. More commonly the edges of strands were lined simply by collagen. Strands were identifiable in sections of the sponge tissue by the presence of an increased number of aligned elongate cells, vast numbers of bacteria, and a much denser collagenous mesohyl in which the collagen fibrils were often aligned in a single direction (Fig. 3A, B). Elongate cells (40–50-μm long and 2.5–4-μm wide) with numerous filopodia were separated laterally by about 10 μm (Fig. 3A). These cells typically lacked conspicuous nucleoli and contained many small vesicles. Other cells in the strands (possibly spherulous cells) were stretched around large vesicles containing an electron-dense material (Fig. 3A). Along particularly dense bands of aligned collagen there were highly elongate cells with a well-developed cytoskeleton (Figs. 3B, 4A). Cells in the strand were often found in the process of phagocytosing bacteria (*e.g.*, Fig. 4B).

The tissue outside of the strands consisted mostly of a comparatively loose collagenous mesohyl containing numerous bacteria, although fewer than in the strands; scattered amoeboid-like cells; and flagellated chambers. The sponge cortex consisted of many spherulous cells containing large inclusions, and some elongate cells embedded in collagen that was not aligned in any particular direction (Fig. 4C).

Strands from other species of *Aplysina* were identical in ultrastructure to those in *A. cauliformis*. Cells in strands that were preserved after they had been forced to recoil at the edges by cutting and tweezing appeared no different than those in strands that had been fixed while still within the sponge. In both cases, cells were elongate when lying beside aligned collagen fibrils and irregularly ovoid when not.

Feeding and insertion of latex beads

In the feeding experiment, the beads were first seen in the flagellated chambers and pinacoderm (Fig. 5a) and later in cells in the mesohyl (Figs. 5B and 2f). Four hours after feeding, beads were found in all the sponge tissues of the middle segment except the cell strands (Fig. 2f). By 2 to 3 days after feeding the beads were concentrated in the tissues around the water canals (Fig. 2g). At this time only a few beads were found in the cell strands. However, 7 to 11 days after feeding, many beads were found in the tip segment, where they were specifically lodged in the cell strands (Fig. 2h); very few were found in adjacent tissues. Closer examination of selected stained sections showed that the beads were within the cells in these strands. At this level of resolution it appeared that the beads were in the elongate cells rather than in the spherulous cells, but the strands with beads were not examined by electron microscopy to confirm this observation.

The relative proportion of beads in the tissue, at the edges of canals, and in the tissue strands over the course of 13 days after latex bead feeding and insertion is shown in Figures 6 and 7. In the feeding experiment (Figs. 6A and 7A) most latex beads in the middle segment were first found in the sponge tissues (triangles) and later at the edges of water canals (squares) during the first week after feeding. The number of beads in the middle segment declined during the second week after feeding. Although many beads were counted in the strands in the middle segment at day 2 (see Table I), these were far outnumbered by those beads that were counted around the edges of the canals and in the tissue at this time. In the second week, 22% of the beads counted in all segments were in the strands in the tip segment 11 days after feeding (Fig.

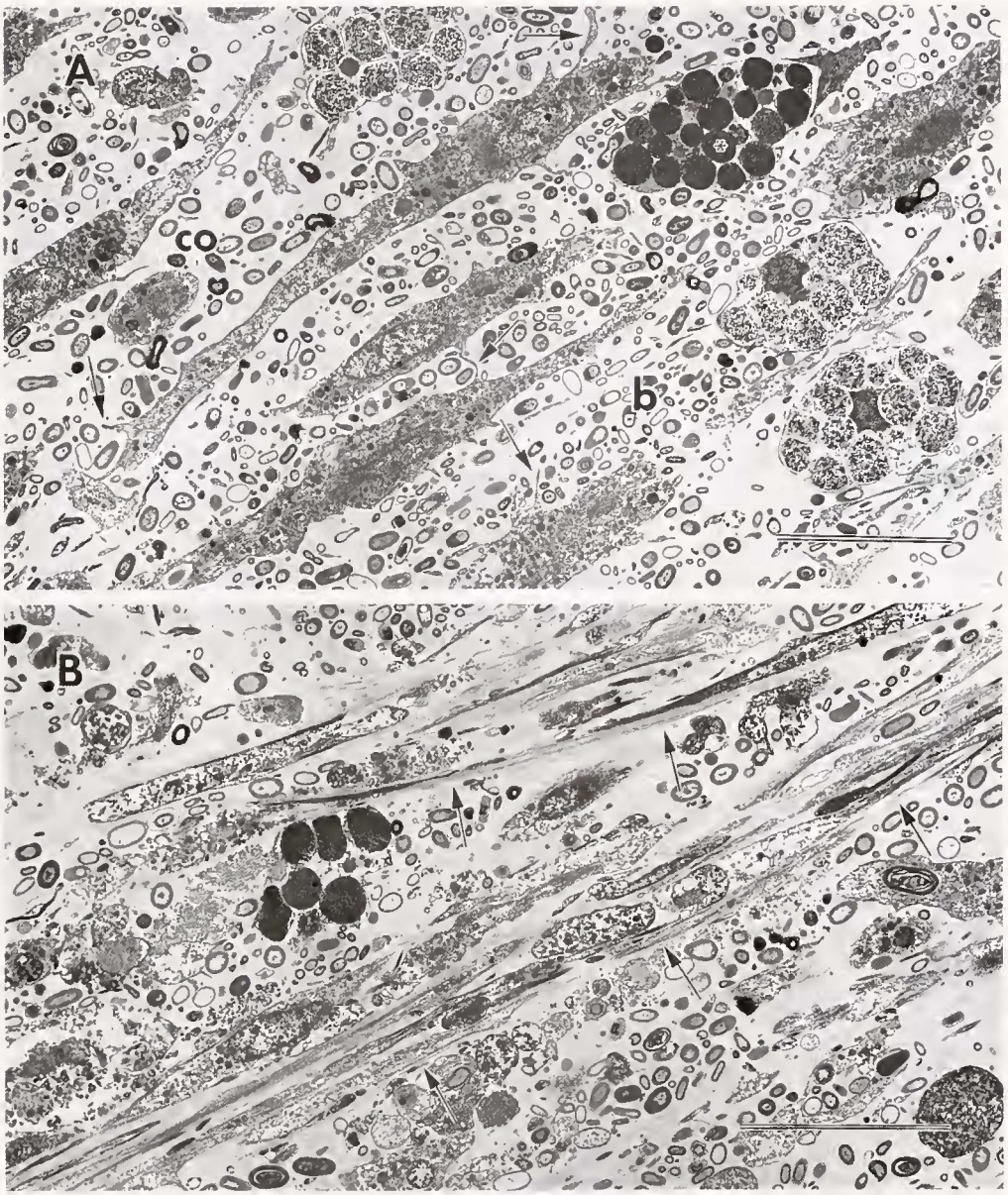


Figure 3. Longitudinal sections of tissue strands from *Aplysina cauliformis* (transmission electron microscopy). (A) A section showing several elongate cells with numerous filopodia (arrows). These cells lie lengthwise throughout the strand within a bacteria-filled (b) collagenous mesohyl (co). Other cells in the strands contain large inclusions of electron-dense material (*). Bar: 10 μm. (B) Some cells in the tissue strands were highly elongate and were tightly aligned within dense bundles of collagen fibrils (arrows). Bar: 10 μm.

6A: tip segment). However, 7 to 11 days after feeding, 95% of all the beads counted in the tip segment alone were in the strands (Fig. 7A). Many beads were found around the water canals in the lower segment in the feeding experiment. A week after feeding, 63% of the beads counted in the lower segment were found in the strands (Fig. 6A: lower segment).

Latex beads that were inserted into the sponge branches about 7 cm from the tip were transported predominantly upward to the tip 2 weeks after insertion (Fig. 6B: tip segment); very few beads reached the segment of the sponge below the insertion point. One day after their insertion, the beads were abundant in the general tissue of the sponge in the middle segment, much as was found in the feeding experiment. After 3 days a large proportion of the beads were found at the edges of the water canals, and a few (5%) were in the tissue strands in the middle segment. Whereas the number of beads counted in the

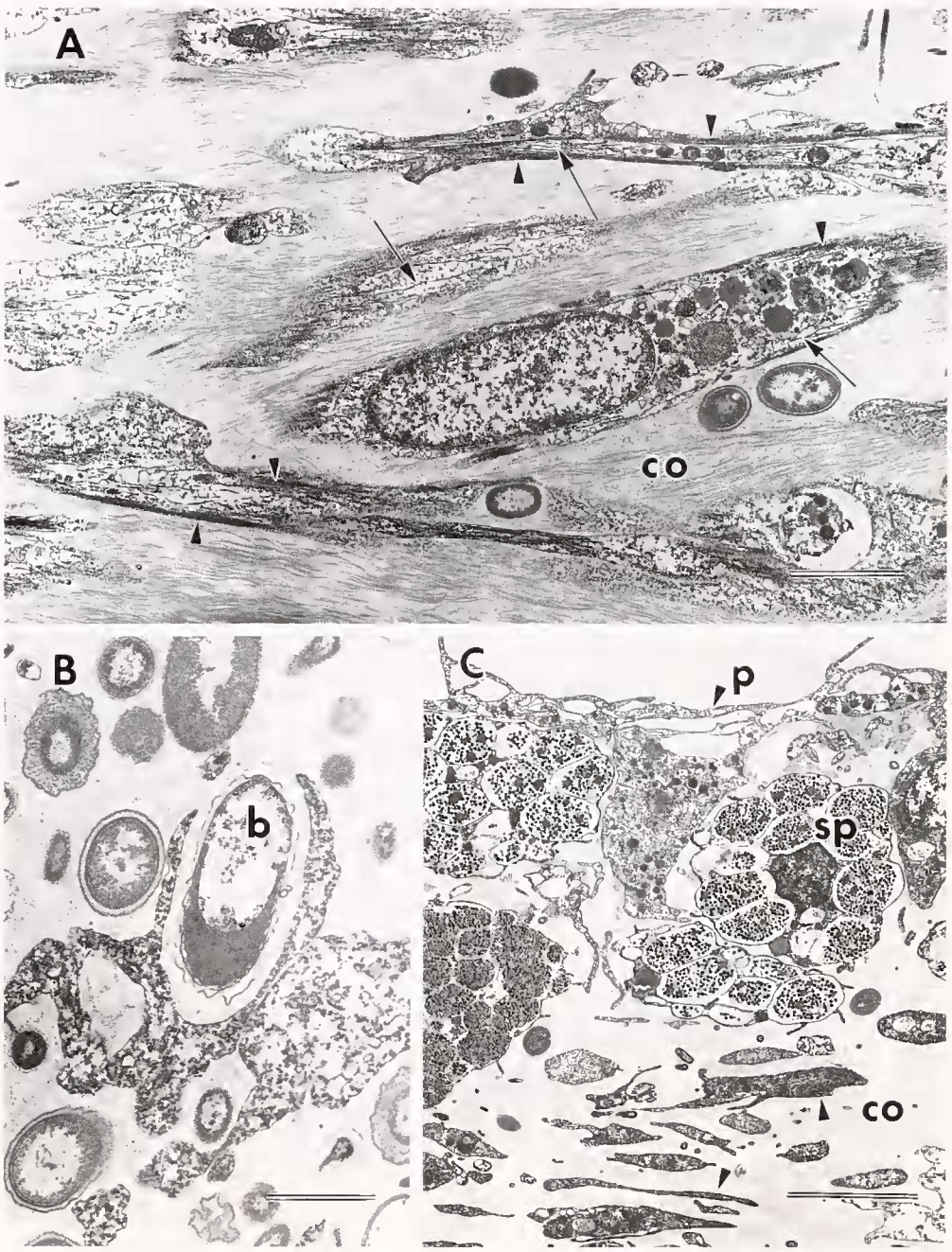


Figure 4. Aspects of the cell strands and the cortex of *Aplysina cauliformis* (transmission electron microscopy). (A) A higher magnification of cells among densely bundled collagen fibrils (co) in strands, showing bundles of electron-dense fibrils, which may be microfilaments (arrowheads), and of microtubules (arrows), in the cells. The microtubules were identified as such from their diameter in high magnification electron micrographs. Bar: 2 μm. (B) An example of a cell within a strand in the process of engulfing a bacterium (b) Bar: 1 μm. (C) The cortex of the sponge. Just inside the dermal layer or pinacoderm (p) are numerous spherulous cells (sp) with large inclusions, and individual elongate cells (arrowheads) lying in a collagenous mesohyl (co). These elongate cells are far shorter than those in the strands and are not in large tracts of aligned collagen fibrils. Bar: 5 μm.

middle segments declined toward the end of the second week, their number in the tip segment increased at this time. After 7 days, 42% of the beads counted in the tip segment were in the strands; 13 days after feeding, however, most beads in the tip segment were in the tissue (Fig. 7B).

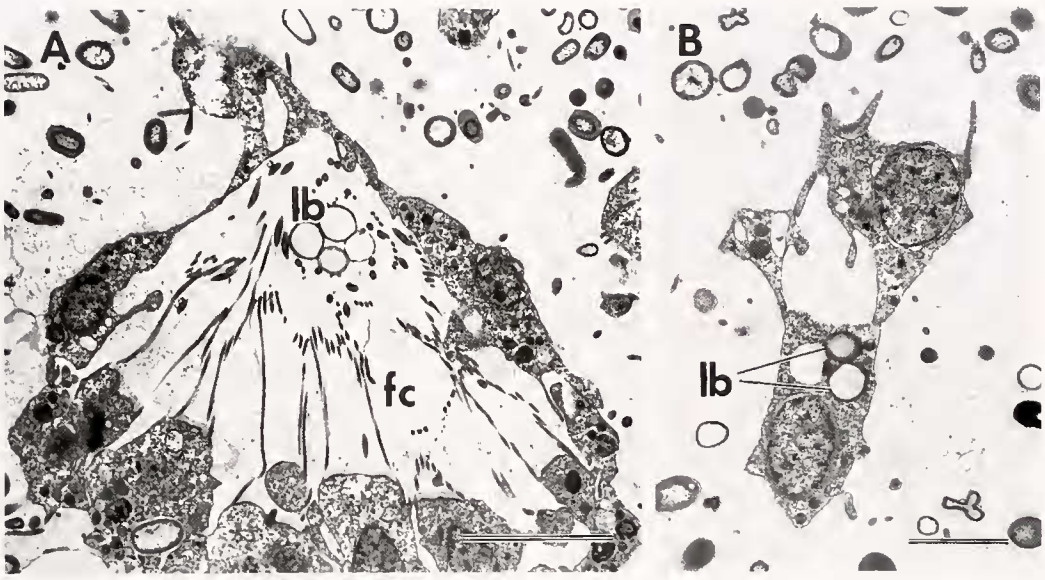


Figure 5. Latex bead uptake by *Aplysina cauliformis* (transmission electron microscopy). (A) Four hours after feeding, latex beads (lb) were found in flagellated chambers (fc) and (B) in ameboid cells in the mesohyl. Bar (A): 5 μm; (B): 2 μm.

Video microscopy

Attempts to record movement of cells in the tissue strands of *Aplysina* were hampered by the opacity of the tissue strands; indeed, only cells at the edges of strands could be monitored by transmitted light microscopy. Consequently, strands that were exposed by cutting the sponge branch longitudinally were illuminated from above, and viewed at a low magnification with a 10× objective lens. The image was magnified by both the video camera and the digital image processor. With this system, highly refractile cells, which may correspond to the spherulous cells shown in Figure 3A, were the most conspicuous element in the strands; the movements of the smaller cells could not be monitored. The refractile cells showed no obvious movement as a group in either direction along the strand. Nevertheless, more than 2 h after filming began on the sponge, several individual cells definitely moved along the strand in both directions at rates of 0.025 $\mu m \cdot s^{-1}$ to 0.04 $\mu m \cdot s^{-1}$ ($n = 8$) (Fig. 8). Thus, the movement was probably not caused by contraction of the strand after wounding.

Discussion

Endosomal tissue strands have not been described in sponges other than the genus *Aplysina*, nor is there any record that a specialized structure is involved in the distribution of particulates in sponges. We have shown here, however, that when latex beads are fed to *A. cauliformis*, they are taken up and transported into the tissue strands; eventually they end up at the tip of the sponge or further down the stalk.

Sponges are well-known to have highly motile cells, whose rate of movement (2 to 21 $mm \cdot day^{-1}$; Bond, 1992)—although slower than that of crawling by *Amoeba*—is comparable to, and even faster than crawling by fibroblasts and neutrophils (Bray, 1992). Furthermore, at the growing edge of sponges, these cells (particularly those of a similar type) often become aligned in tracts (Bond and Harris, 1988). Similar tracts of cells, sometimes referred to as cell "cords," have been described in various demosponges where their suggested role has been in growth (Brien, 1976), regeneration (Lévi, 1960), and remodeling (Diaz, 1979). In each of these cases, the primary role of the cell cords was thought to be in skeletogenesis (Simpson, 1984), as demonstrated by Teragawa (1986) for the keratose sponge *Dysidea etheria*. However, none of these tracts or cords of cells is so permanent a structure within the sponge, nor so widespread and morphologically uniform within a genus, as the endosomal tissue strands in *Aplysina*. Nor can any of those tracts be so readily extracted from the rest of the tissue as can *Aplysina*'s tissue strands.

The bead uptake experiments here show that the strands are involved in transport of materials taken in during feeding. Most of the beads that were fed or inserted into the sponges were excreted within a week; relatively few ended up in the strands, which suggests that most of the food taken in by flagellated chambers and the adjacent pinacoderm is probably distributed to cells locally, and wastes are probably expelled from the same area. However, a role for the strands as transport pathways is supported by the observation that a substantial proportion of beads were in the strands in the tip segment 2 weeks after

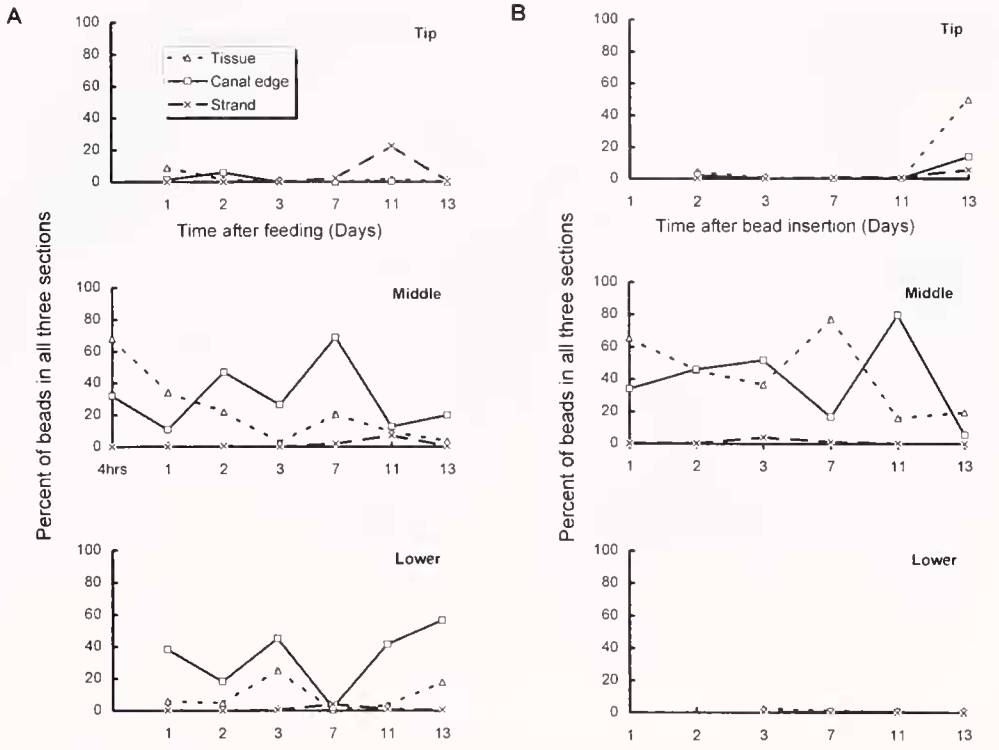


Figure 6. Latex bead experiments. Percent of the total number of beads counted in all sections of all three segments (Tip, Middle, and Lower) at each time interval, in the tissue (triangles), at the canal edges (squares), and in the strands (crosses): (A) feeding; (B) insertion. See text for explanation.

the middle portion of the sponge was fed. The beads found in the strands 2 days after the sponges were fed were far outnumbered by the beads counted around the canal edges or in the tissue (see Table I). The delayed appearance of the beads in the tip segment 7 days after feeding is best explained by the time required for the cells transporting the beads to crawl first to the strands and then along the strands to the tip, where they accumulated. That downward transport also occurs is suggested by the number of beads found in strands in the lower segments a week after feeding, and later around the water canals. The beads found around the canal edges in the lower segment may have been transported down and then moved to the canals for excretion, or they may have been reingested after excretion from the middle section.

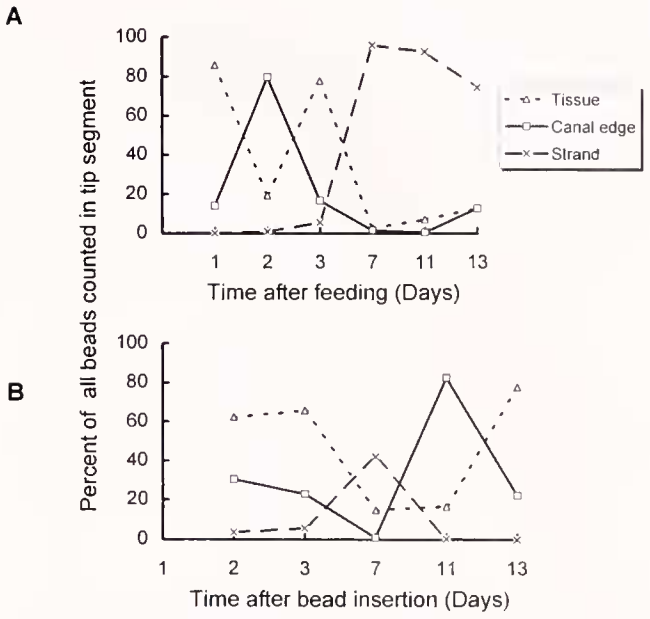


Figure 7. Latex bead experiments. Percent of all the beads counted in the tip segments only: (A) feeding; (B) insertion. See text for explanation.

Aplysina fulva has been shown to grow rapidly, at an average rate of 2 cm · month^{-1} (minimum 0.2 cm · month^{-1}, maximum 15 cm · month^{-1}; calculated from Wulff, 1990). For this rate of growth, rapid translocation of materials to the tip would be necessary despite the ability of flagellated chambers at the tip to take in and distribute nutrients locally. Downward transport might supply regions of the sponge that possibly feed less and function primarily as a stalk. Video microscopy showed that individual cells moved within strands for distances of 30 μm or more at a rate of 2.2 to 3.5 mm · day^{-1}. At this rate of movement, material could be transported 5 cm in 2 weeks, if transported more or less in a straight line. The large numbers of bacteria in the strand might support the energy require-

Table 1

The number of latex beads counted in the tissues, around the water canals, and in the strands of each segment of fed branches of Aplysina cauliformis

Collection period	Segment[a]	Number of beads: In each segment region[b] Tissue	Canal edge	Strand	Total per segment	Total per day (all segments)	Percent of beads in each segment/day
day 1, 4 h	Middle	20,000	9,400	7	29,407	29,407	
day 1, 24 h	Lower	572	3,765	20	4,357	9,792	44.5
	Middle	3,348	1,060	6	4,414		45.1
	Tip	876	144	1	1,021		10.4
day 2	Lower	5,116	19,400	65	24,581	106,472	23.1
	Middle	23,400	49,950	548	73,898		69.4
	Tip	1,554	6,366	73	7,993		7.5
day 3	Lower	2,890	5,219	48	8,157	11,536	70.7
	Middle	319	3,042	0	3,361		29.1
	Tip	14	3	1	18		0.2
day 7	Lower	78	355	766	1,199	18,554	6.5
	Middle	3,806	12,753	346	16,905		91.1
	Tip	12	7	431	450		2.4
day 11	Lower	1,090	12,779	290	14,159	30,698	46.1
	Middle	2,843	3,914	2,212	8,969		29.2
	Tip	535	37	6,998	7,570		24.7
day 13	Lower	1,148	3,617	39	4,804	6,394	75.1
	Middle	247	1,274	30	1,551		24.3
	Tip	5	5	29	39		0.6

[a] Lower, Middle, and Tip segments of each sponge branch fed latex beads.

[b] Beads were counted in 10 sections from each segment of sponge (Lower, Middle, Tip) each day, and were noted as being in three regions of the segment: Tissue (flagellated chambers and associated tissues); Canal edge (cells specifically around the edges of water canals); and Strand (only within cell strands).

ments of so many moving cells, and this notion is substantiated by the ample phagocytosis of bacteria seen in cells in the tissue strands.

The presence of tissue strands in tube- and stick-forms of *Aplysina* suggests that the mechanism of transport is efficient for growth regardless of sponge form, and indeed has been maintained by at least four species in this genus. Whether more distantly related verongiid sponges also possess tissue strands or similar structures for nutrient transport or other functions is not known.

Feeding in sponges has been well-documented, and with the exception of the two examples cited in the introduction, the Cladorhizidae and the Hexactinellida, particle uptake in sponges occurs at the choanocytes in the flagellated chambers or at the pinacoderm-lined incurrent canals. Food is transferred *via* food vacuoles from the choanocytes to amebocytes, which deliver the nutrients locally to other cells, and wastes are excreted *via* the excurrent canals (Kilian, 1952; Weissenfels, 1976; Willenz, 1980; Imsieke, 1994). Directional translocation of cells and nutrients occurs in gemmule formation (Rasmont and de Vos, 1974) and during oogenesis (Fell, 1983), but this transport occurs over very short distances. Hexactinellid sponges possess perhaps the longest transport pathway known in the Porifera, but translocation is intrasyncytial and occurs through a highly dynamic three-dimensional network of reticulated tissue, rather than along fixed pathways (Wyeth *et al.*, 1996; Leys, 1998).

Although sponges have not previously been shown to possess specific transport pathways for nutrients, all invertebrates have developed means of distributing nutrients. Many higher invertebrates such as ascidians, most echinoderms, and crustaceans have a well-developed fluid circulatory system with numerous types of hemocytes which, in addition to their many other functions, transport nutrients. Others invertebrates, however, move food around in a much slower manner: branching corals translocate nutrients to the tip of branches for tip growth (Buchsbaum Pearse and Muscatine, 1971); gorgonians have cells that travel through the stem canals and solenia, a collagenous filled mesohyl, to deliver nutrients to the other tissues of the animal (Murdoch, 1978); fixed paren-

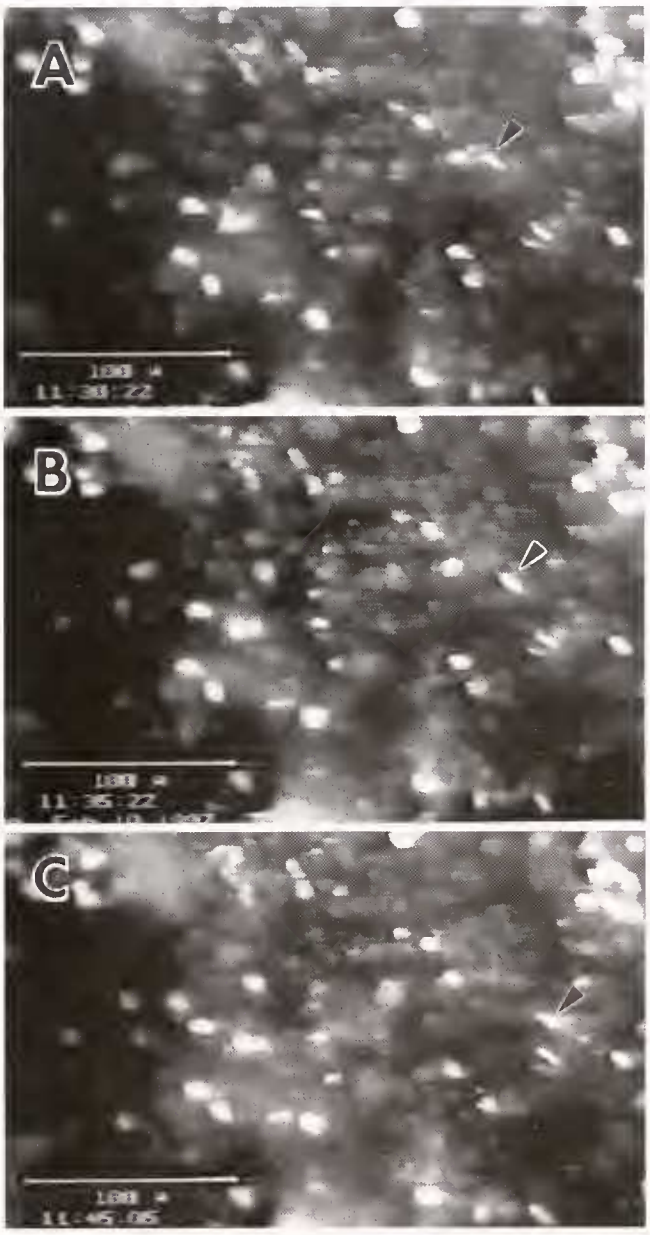


Figure 8. Cell movement in tissue strands (video microscopy). A highly refractile cell (arrowheads) was traced as it moved for 30 μm along a tissue strand at 0.033 $\mu m \cdot s^{-1}$. Time of frames: (A) 0 min; (B) 5 min; (C) 15 min. Bar: 100 μm.

chymal cells in turbellarians are thought to act as a sort of stationary intracellular circulatory system (Pedersen, 1961); and acid phosphatase staining has shown that amebocytes in the hemal lacuna of crinoids (again, a collagen-filled pathway) are involved in digestion and transport of nutrients (Heinzeller and Welsch, 1997).

We cannot rule out a role for the tissue strands in contracting the ostia and water canals to control water flow as suggested for the elongate cells in *Verongia* (Vacelet, 1966); nonetheless, there is little evidence to support this hypothesis. Although some cells in the strands have what appears to be a highly developed actin cytoskeleton, and the strands recoiled gently after being cut, none of the strands were wrapped around the water canals or ostia sufficiently to be able to reduce water flow if contracted. Furthermore, localized patches of elongate cells were present in valve- or sphincter-like structures around the water canal system, and these quite possibly function in controlling water flow through the sponge as suggested by Vacelet (1966), and by Reiswig (1971) for those sponges in which flow is not controlled by flagellar activity. The facts that video microscopy did not reveal all cells in the strands to be moving, and that not all cells in the strands are elongate, could also be taken as evidence against the transport hypothesis. But the thickness (100 μm) of the strands allowed only a few cells at the edges of strands to be observed at high magnification by video microscopy. Despite the low magnification and use of epi-illumination, a few of the highly refractile cells could be clearly seen to travel for significant distances along the length of the strand.

It could also be true, however, that not all the cells in the strand transport material. Thin sections of the strands showed that only some cells in the strands had a highly developed actin cytoskeleton and were aligned along densely bundled collagen fibrils. Other cells were clearly elongate with filopodia at either end, but were not associated with the densely bundled collagen fibrils. Fibroblasts in culture orient themselves along grooves in culture dishes (Carter, 1967; Clark *et al.*, 1980), and on collagen substrates they take on a bipolar spindle morphology with filopodia at either end (Elsdale and Bard, 1972). Furthermore, Harris (1987) has demonstrated that the forces exerted by fibroblasts in culture are far greater than is necessary for cell crawling, and that the prime function of these cells is to bundle and align collagen to prepare the path for migratory cells (Stopak and Harris, 1982).

An analogous situation could exist in *Aplysina* strands. We suggest that the role of the highly elongate cells with a well-developed actin cytoskeleton is primarily to bundle and align the collagen fibrils, creating a pathway that cells transporting nutrients can recognize and follow, thereby allowing the rapid transport of materials either to the tip or to the base of the sponge (Fig. 9). In this fashion, apoplastic nutrient transport by ameboid cells has become specialized in the genus *Aplysina* to the extent that these sponges have a differentiated structure that parallels fluid transport systems in other animals, but is a typically novel poriferan solution.

Acknowledgments

We thank the director and staff at the Bellairs Research Institute (BRI) of McGill University, in Barbados, for the use of facilities while we were conducting the field work

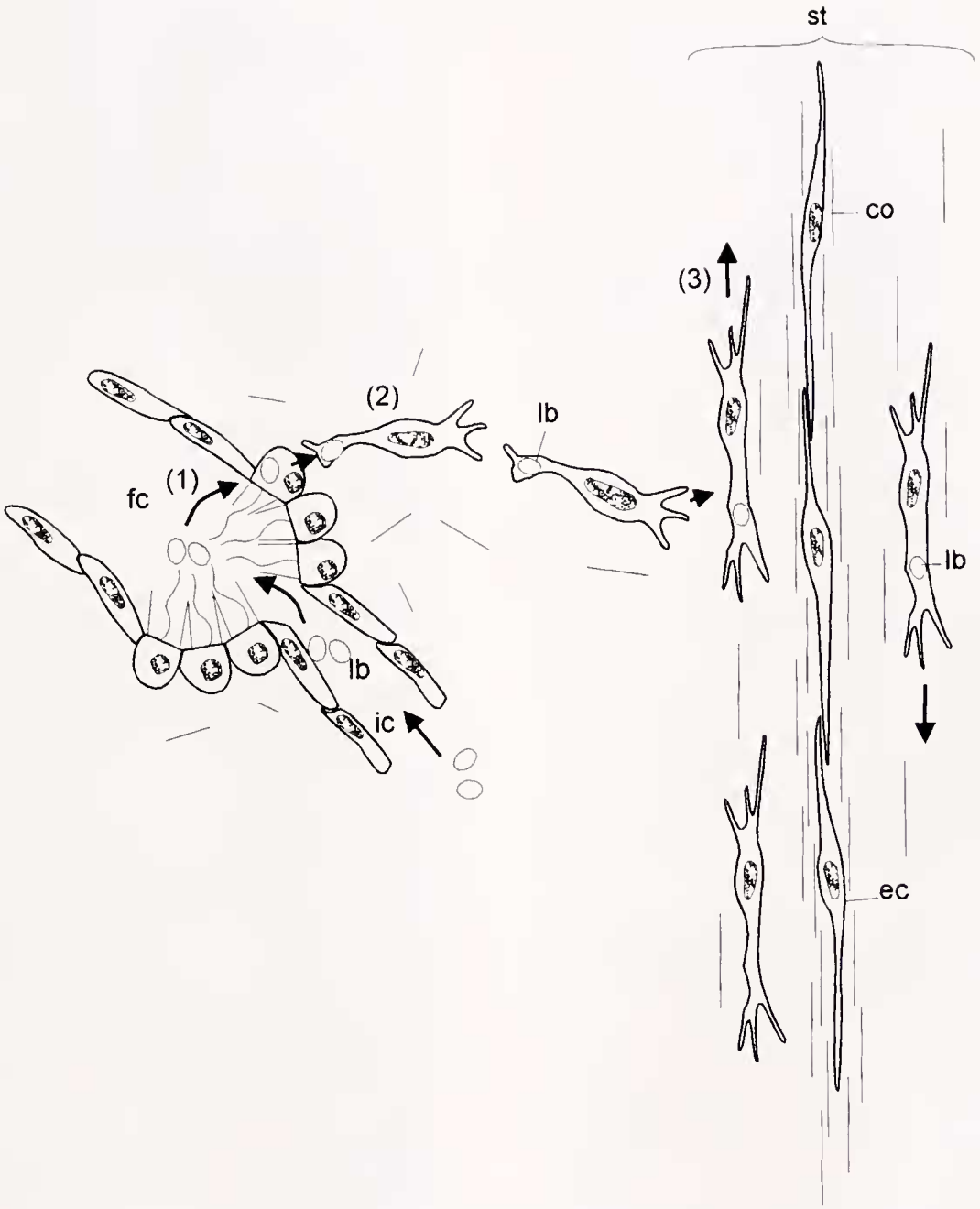


Figure 9. Diagram of the proposed route taken by latex beads (lb) that were fed to *Aplysina cauliformis*. Beads are taken in *via* the incurrent canals (ic) to the flagellated chambers (fc), where they are ingested (1) by choanocytes. The beads are then transferred to wandering amoebocytic cells (2), which encounter strands of elongate cells (ec) and highly bundled collagen fibrils (co). The amoebocytic cells follow the direction of the collagen, thereby transporting the latex beads up and down the strand (3) as shown by the arrows.

for this study. We also thank L. Verhegge, M. Tsurumi, and G. O. Mackie for comments on the manuscript. This research was supported by a Commander C. Bellairs Post-doctoral Fellowship from BRI and McGill University to SPL, and by a research grant from the Natural Science and Research Council of Canada (OGPOO 1427) to G.O.M.

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